Reproductive pattern and histological profiling of maturity stages in *Tenualosa ilisha* of Chattogram Coast, Bangladesh

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Roll No: 0119/08

Registration No: 707

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Marine Bioresource Science

Department of Marine Bioresource Science

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

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This is to certify that we have examined the above Master's thesis and have found that it is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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AUTHORIZATION

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(Mahfuja Binte Malek)

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ABSTRACT

Hilsa is an anadromous fish belongs to the family Clupeidae known as 'Indian shad' in Bengali. *Tenualosa ilisha, Hilsa kelee* and *Hilsa toli* are the three species found in the Bay of Bengal, although *Tenualosa ilisha* is the most common hilsa fish caught in our nearby region. This study is done on the reproductive pattern and histological profile of *T. ilisha* in the Chattogram coast, Bangladesh. For analyzing the result length-weight relationship, condition factor, relative condition factor, oocyte diameter, fecundity and histological stages were identified by collecting fish sample throughout the year (February, 2019 to January, 2020). The length-weight relationship based on pooled data was $W = 0.269874 * TL^{2.6351}$ ($R^2 = 0.682$), the logarithmic equation being $\log W = -1.3098 + 2.6351 \log TL$. The 'b' value of the length-weight relationship indicates the negative allometric growth ($b<3$) of *T. ilisha*. It was found that, the mean of condition factor (K) was minimum (0.9905±0.0859) during May and maximum (1.5343±0.2438) during September, again the mean relative condition factor (K_n) was minimum (0.1404±0.0126) during May and maximum (0.2177±0.0286) during September. Highest GSI value (13.57±1.618) for female was found in January whereas lowest value of GSI (1.698±0.4968) was observed in March. Maximum oocyte diameter was found in January (890.6±122.09 μm) and minimum oocyte diameter was found in March (220.9±35.67 μm). Fecundity was counted by gravimetric methods and was found to vary from 96718 to 117435 with a mean value of 109605±1635 eggs. Throughout the year, six different gonadal developmental stages were found. According to this study, ripe stage was identified in the month of January and in other months it passes different gonadal stages like primary vitellogenic, secondary vitellogenic, maturing, spent and regressing. The findings will provide to a better understanding of the stages of gonadal development of *Tenualosa ilisha* and the maintenance of the catching period in accordance with these stages.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study:

The fisheries sector is one of the most productive and dynamic industries in agricultural economy of Bangladesh with enormous potential for future growth. Bangladesh has a diverse range of fisheries resources that may be divided into two categories: inland and marine (Shamsuzzaman *et al.*, 2020). According to the research, fish is the predominent protein source in the Bangladeshi diet, accounting for around 60% of total animal protein. Overall fish consumption is estimated to be 62.58 grams, which is more than their daily recommended intake for protein (60gm) (BBS, 2017). In an agro-based culture, fisheries have historically played a significant role in the national economy as a major source of animal protein, job opportunities, food security, foreign revenue and socio-economic growth (FRSS, 2017).

Hilsa is an anadromous fish belongs to the family Clupeidae known as ‘Indian shad’ in Bengali which is known as Bangladesh’s ‘national fish’ (Islam *et al*., 2016). Hilsa is a widespread fish that lives mostly in marine and estuary environments. The fish may be found in the Arabian Sea, Red Sea, Vietnam Sea, China Sea, Bay of Bengal, and Persian Gulf's marine environment. The Padma, Jamuna, Meghna, Karnaphuli and other coastal rivers of Bangladesh, the Satil Arab, the Tigris and Euphrates of Iran and Iraq, the Indus of Pakistan, the Irrawaddy of Myanmar and the rivers of Eastern and Western India are all part of the estuary ecosystem (Mome, 2007). *Tenualosa ilisha*, *Hilsa kelee* and *Hilsa toli* are the three species found in the Bay of Bengal, although *Tenualosa ilisha* (Fig. 01) is the most common Hilsa fish caught in this vicinity.

Fig. 01: Hilsa Fish (*Tenualosa ilisha*)

As a consequence of the government's tight measures, the country's entire Hilsa output in the 2020-21 fiscal year was 5.50 lakh tonnes. Bangladesh contributes 86% of the overall Hilsa catch, with the remaining 14% coming from India and Myanmar (Miah, 2015). Hilsa contributes 12% of Bangladesh's total fish production. Hilsa accounts for 1.15% of Bangladesh's Gross Domestic Product (GDP) and contributes significantly to the country's economy by generating foreign cash (FRSS, 2022). It is immensely popular in Bangladesh and South Asia, not only because of its economic benefits, but also because of its distinct flavor (Kumar and Banerjee, 2012). The Hilsa fishery is directly or indirectly responsible for the livelihood of a huge number of people in our country (Prakash *et al.*, 2020). Approximately 0.5 million people rely on Hilsa fishing for a living, while another 2 million people rely on Hilsa fishing indirectly through industries like as transportation, marketing, and processing (Islam *et al*., 2016).

Hilsa migrates from marine water to fresh water through the estuary due to spawning (Fig. 02). It spawns in a definite period of the year, however the Bengali months of Ashwin-Kartik (September-October) are the main spawning months due to the full moon phase. During this time, the majority of the brood hilsa has been caught in the country's spawning areas. Hilsa begin their upstream migration in January-March, right before the monsoon (rainy season), and begin spawning in July-September, when the rains and floods occur. The Hilsa migrate upstream from the Bay of Bengal to spawn two to three years into their life cycle (Rahman *et al.*, 2017).

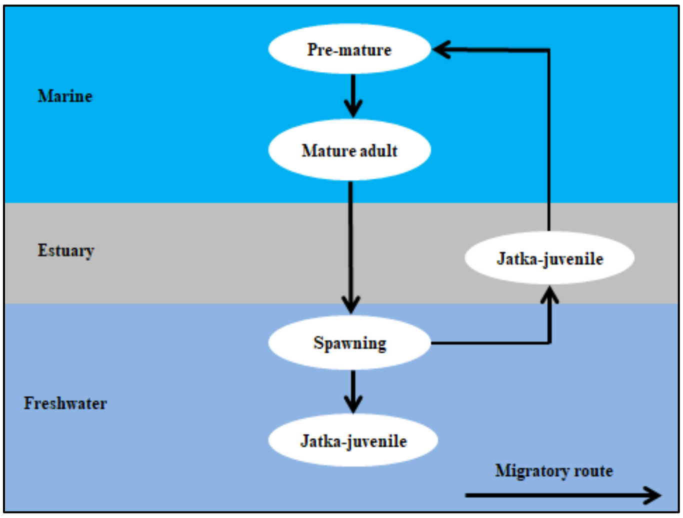


Fig. 02: Movement pattern of *Tenualosa ilisha* into different habitats

The government of Bangladesh has made steps to conserve brood and juvenile hilsa in the nursery and spawning areas, recognizing the economic and nutritional value of the species. Government of Bangladesh have prepared Hilsa Fishery Management Action Plan (HFMAP) and imposed ban on indiscriminate catch of hilsa during the specific time of the year. The Department of Fisheries (DoF) imposes a fishing restriction every year with the assistance of law enforcement organizations such as the Coast Guard, Bangladesh Navy, Naval Police and RAB (Sunny *et al*., 2017).

1.2 Objectives of the research:

The specific objectives of this study are:

- To determine the reproductive biology of *Tenualosa ilisha* from Chattogram coast.
- To find a pattern of different histological stages of *T. ilisha* with monthly variation of the collected sample.

1.3 Research questions:

- When does *T. ilisha* gonad development starts?
- How do gonadal stages varies according to the monthly changes?

CHAPTER TWO

REVIEW OF LITERATURE

Length-weight relationship studies are an important tool for understanding the basic biology of fish. A detailed check at the condition throughout different months can give definite hints about the breeding seasons, whereas evaluating the conditions at different body lengths can give significant information about the maturation and spawning the life cycle of the fish. This chapter is about the review of length-weight relationship, GSI, cyclic changes in the gonadal development, fecundity of *T. ilisha* species. The following data were gathered in order to plan the current study and validate the new findings.

2.1 Distribution of Hilsa

Haroon (1998) identified the following five spawning grounds for Hilsa in Bangladesh : 1) In the Noakhali district of the Chattogram division, to the south and around Moulavir Char of Hatia Island, and along the Hatia canal with the Bay of Bengal, 2) Extending up towards the northwest of Moulavir Char underneath the Bhola from southest of Hatia to the east and southeast of Monpura Island, 3) Junction of lower stretches of the Meghna river with the Bay of Bengal, 4) South of Char Fasson of Bhola and the surrounding area, and 5) Copulation of the Shahbazpur River with the Bay of Bengal. Hilsa also spawn off the coast of Sandwip, in the Sandwip and Hatia channel, which connects the bay to the Chattogram division. During the monsoon season, de Graaf *et al.* (1999) discovered a high density of Hilsa larva in the upper strata of the river Lohajang near the Tangail beach. So far, two large nursery grounds have been identified, one in the riverine area and the other in the coastal area. The Meghna river has the greatest riverine nursery ground, stretching from Shatnol (Louhojang-Mawa Gazaria, Munshiganj) in Dhaka division upstream to Nilkamol in Chandpur and downstream to Hajimara-char Alexandar in Lakshmipur in Comilla division (Milton, 2010). Juvenile Hilsa (jatka) are abundant (Fig. 03) in these nursery grounds from January to May, with a peak in March-April. The coastal nursery ground stretches south of the Sundarbans from Kuakata (Patuakhali) to Dublar Char (Khulna) **(**Haldar, 2002**).** Ilisha, Karkhana, Pyra, Kirtonkhola, Tetulia, Bishkhali, Shabazpur channel, Arial kha, Dharmagonj and Andharmanik are the primary coastal rivers used by Hilsa as nursery grounds in Barisal and Bhola districts. Hilsa's nursery grounds also include huge rivers in and around the Sundarbans, as well as other coastal islands (Hossain *et al*., 2016).

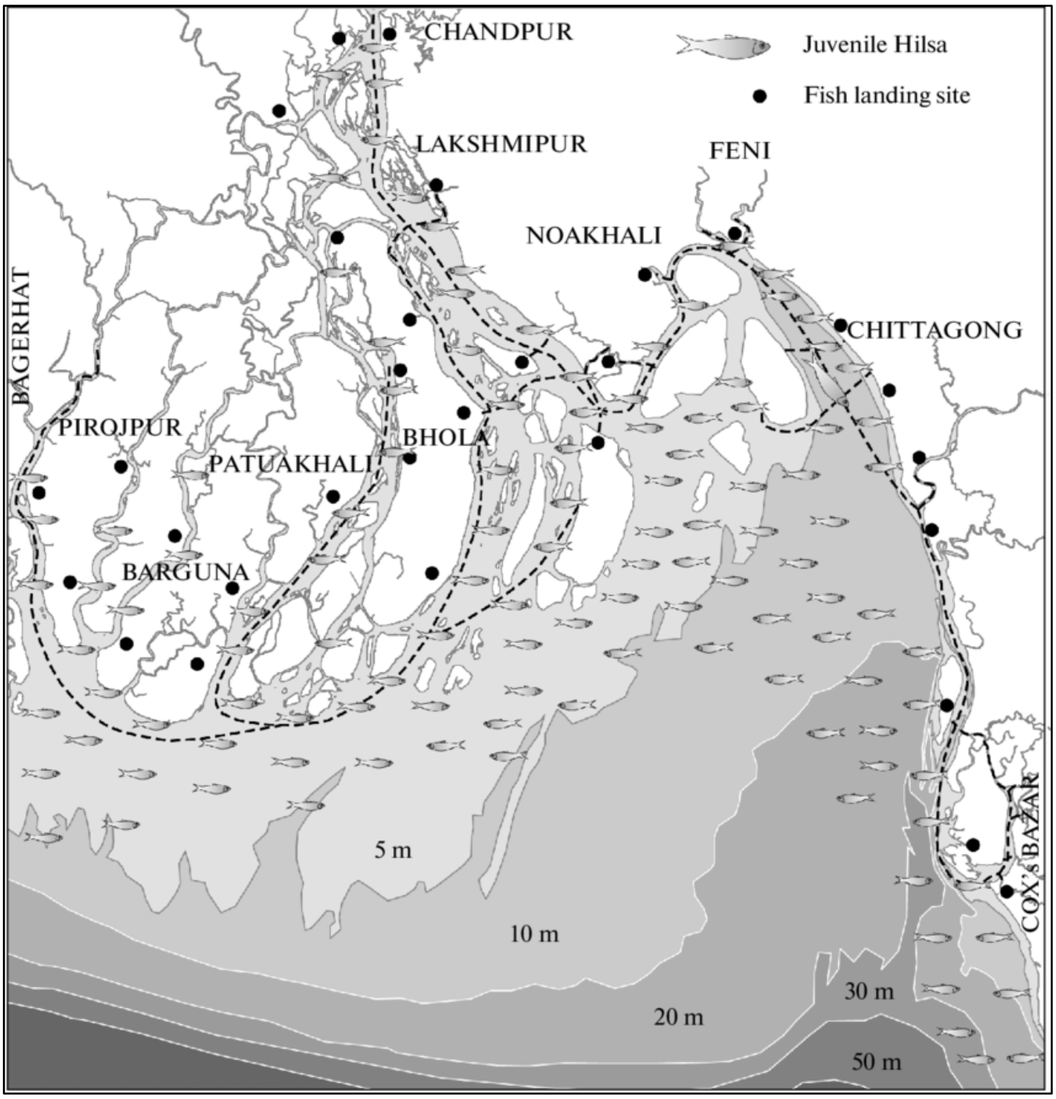


Fig. 03: Distribution of juvenile Hilsa in the rivers, eatuaries, and coastal waters of Bangladesh (Hossain *et al*., 2014)

2.2 Length-weight relationship

The length-weight relationship of *Tenualosa ilisha* during spawning migration in the Shatt al Arab River and Southern Al Hammar Marsh was investigated by Almukhter *et al*. (2016) from April 2013 to March 2014. Gillnets and landings were used to collect a total of 1456 specimens. The total length of the specimens obtained throughout the migration season ranged from 50 to 460 mm (TL). For both sexes, the monthly variation in mean length exhibited two distinct peaks, the first in February-March and the second in September-November. Total length of females ranged from 90-460 mm, with an average of 262.04 (±64.6 SD) mm and a body weight range of

8.0-1263 g, with an average of 241.79 (±106 SD) g. Males had a total length range of 50-400 mm, with mean of 245 (±56.7 SD) mm and a body weight range of 3.5-690 g with an average of 178.89 (±67.7 SD) g. The largest weight of female fish and male fish was found in June.

Roomiani *et al*. (2014) carried out a study in the Persian Gulf and rivers of Khouzestan Province of Iran were analyzed. A total of 486 fish were sampled by gillnet from the landing center of Arvand (AR) and Bahmanshir (BR) rivers during the period of April, 2010 to September, 2010. In PG, the average length of both male and female *T. ilisha* was 365.82 and 386.76 cm, AR and BR, 363.82, 381.14 and 391.00, 403.13 cm, respectively. In PG, the average weight of both male and female *T. ilisha* was 523.37 and 646.85 g and AR and BR, 588.67, 698.51 and 596.07, 723.83 g, respectively. The Length-Weight relationship was calculated for PG as W= 1.469L2.687, AR and BR as W=2.188L3.167 and W=1.850L2.947, respectively.

The study on the length-weight relationship of *Tenualosa ilisha* in Meghna River, Bangladesh was conducted by Flura *et al*. (2015). In this study the length-weight relationship of hilsa were investigated using monthly samples collected from the Meghna River in Chandpur, Bangladesh, from January to December, 2013. There were 517 hilsa specimens collected with 171 males and 346 females. Male and female parameter values for the equation $W=aL^b$ representing the relationship between total body weight (BW) and total length (TL) changed monthly. The generalized length-weight relationship was fitted with the pooled data of all monthly samples for male and female separately which were $BW=0.01TL^{3.040}$ (R^2=0.902) and $BW= 0.008TL^{3.078}$ (R^2=0.822) respectively. The results revealed that all length-weight relationships were highly correlated (r>0.891).

The total length of female Hilsa was measured in the study conducted by Akhter *et al*. (2022) from January to December, 2015 in lower Meghna Estuary, Bangladesh. Different sizes of females were considered for the present study. The highest total length was found in July and the lowest in January. In the present experiment, different size group female Hilsa were sampled and their weight was ranged from 420.00g to 1472.00 g. The highest body weight was observed in September and the lowest in January.

2.3 Reproductive biology and spawning pattern

Almukhter *et al*. (2016) estimated the highest GSI values in April (9.5±4) in female and male had the highest GSI values in February (2.5±4). For both sexes, there were two separate peaks. The first peak occurred between March and June, while the second occurred between August and October. This finding revealed that the spawning season lasted from March to November, with discrete spawning peaks at the start and end of the migration. Female GSI has gradually decreased from the estuary to the marshes in terms of spatial distribution. Macroscopic observations of gonads showed six maturity stages. The geographical distribution of maturity stages revealed that hilsa shad spawned in all Shatt Al Arab River stations. The absolute fecundity ranged from 223760-1477880 eggs for fishes with total length range of 200-460 mm and mean weight range of 247.3-891.7 g.

The monthly fluctuations in both sexes Gonadosomatic index (GSI) were extremely noticeable which was investigated by Roomiani *et al*. (2014). Male and female highest readings in Persian Gulf were observed in April. Maximum male and female values were observed in June and May, respectively, in Arvand (AR) and Bahmanshir (BR). The maturation season in AR and BR begins in March, while spawning begins in April and July in AR and August in BR, according to changes in the GSI values.

Flura *et al*. (2015) found the GSI values for female ranged from 0.12 to 24 over the course of the study. The GSI of specimens obtained in March, April, May and August ranged from 0.12-11.06, 2.46-13.7, 0.18-14.5 and 0.68-13.6 respectively. Medium and large GSI values ranged from 5.97-18.97, 1.6-24, 5.28-10.71 and 5.08-16.67 for female fish taken in September, October, November and December 2013. Females GSI levels appeared to have three peaks, which occurred in February, June and October. The highest mean GSI value was calculated in October. The spawning season of *T. ilisha* was largely allocated to October according to this study.

According to the study of Akhter *et al.* (2022) monthly mean GSI values of females ranged from 6.36±0.69 to 15.02±1.33. The lowest mean GSI value was found in December and the highest GSI value was in October. From the histological observation of the ovary, early perinucleolar stage, late peri-nucleolar stage, yolk vesicle stage, yolk granule stage, pre-mature and mature stages were identified. The highest percentage (75%) of mature oocytes and peak breeding season were observed in October and the breeding season continues from October to November.

Das *et al*. (2022) driven a study where samples were collected from Meghna River, Chandpur, from July 2012 to June 2013 and Tentulia River, Barisal from June 2013 to June 2014. The mean GSI value for both female Hilsa of the Meghna River was found to be varied from 4.84±0.63 to 15.31±1.17 with the highest value in October. In the case of the Tentulia River, the mean GSI value for female Hilsa was found to be varied from 6.33±0.39 to 15.03±0.37 with the peak in October. GSI result of this study suggested that Hilsa has a prolonged spawning season, but major spawning takes place in October–November, with a distinct peak in October, indicating the peak spawning season for Hilsa in Bangladesh.

CHAPTER THREE

METHODOLOGY

This chapter deals with the methods that are followed and materials that are used to achieve the objectives of the study. Methodology is an essential and integral part of any research. In this study, a scientific and logical methodology has been followed. The present study was aimed to determine the gonadal stages of *T. ilisha*. The study was based on laboratory work and data were collected for the interpretation of results. To characterize the gonadal stages of *T. ilisha* the following procedures were followed:

3.1 Study area and sampling stations: The research was mainly based on the availability of fish species from Clupeidae family in Chattogram coastal area. For this reason Chattogram coastal area was the main focused area of the research. To make the sample collection easier the whole study area was segmented into three selective sampling stations (Fig. 04).

Sampling station 1 (Patenga, Chattogram): In the hub of Chattogram the new Fishery Ghat region is the place of available fish species having geographical location as the latitude $22^0$32’97.36”N and longitude $91^0$84’58.20”E. This station covers a wide area of Chattogram coast including Patenga sea beach region, Pathoarghata, fishery ghat (New and old), other adjacent fish landing sites of Chattogram.

Sampling station 2 (Kattoli coast, Chattogram): Adjacent to Sagorika beach, Halishohor, Chattogram including Foillatoli Bazar, Bectech Bazar, Kornel Hat Bazar were investigated under this sampling station 2. The positioning of this station is latitude $22^0$34’46.15”N and longitude as $91^0$77’87.25”E.

Sampling station 3 (Cox’s Bazar): Sampling station 3 includes the region of BFDC Landing Center, coastal sites of CVASU field station and other adjacent coats of Cox’s Bazar. The geographical location of station 3 is $21^0$44’53.36”N latitude and $91^0$97’35.1”E longitude.

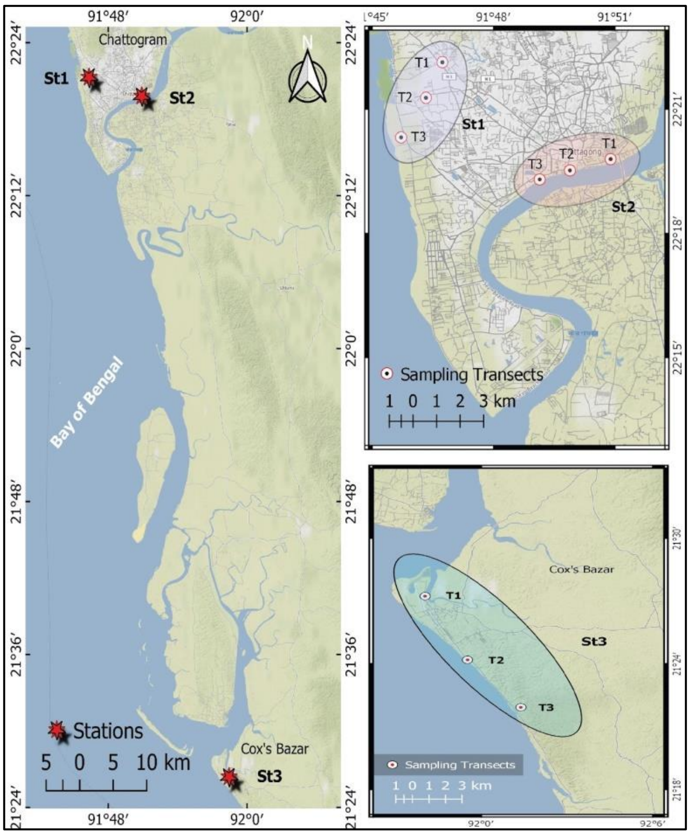


Fig. 04: Sampling stations: a) Patenga, Chattogram b) Kattoli, Chattogram and c) Cox's Bazar

3.2 Sampling period and sampling frequency: The sampling of fish was carried out for a period of one year from February 2019 to January 2020 by regular visits to the sampling stations at monthly intervals. But in this period June, July and October were the ban period for fishing. Sampling from each sampling station was done at the same date of every month. All samplings of a month were also done maintaining 1-2 days interval from one sampling to another sampling.

3.3 Sample collection and transportation:

The fish specimens were collected by following "Simple Random Sampling" method. During sampling the fishes with fresh appearance and having all fins and scales were taken as samples. 3-5 individuals were collected from each sampling stations and 9-10 individuals were collected as sample in every month. Fishes which showed fresh and desired size were considered as sample species which were immediately stored in the ice box with ratio of 1:2 samples and ice, transported to the Oceanography

Laboratory, Chattogram Veterinary and Animal Sciences University, Chattogram for further study (Fig. 05)

Fig. 05: Samples for laboratory analysis

3.4 Recording of length-weight data and determination of length-weight relationship

Total length and total body weight of each fish was measured by using a measuring scale and an electrical balance respectively (Fig. 06).

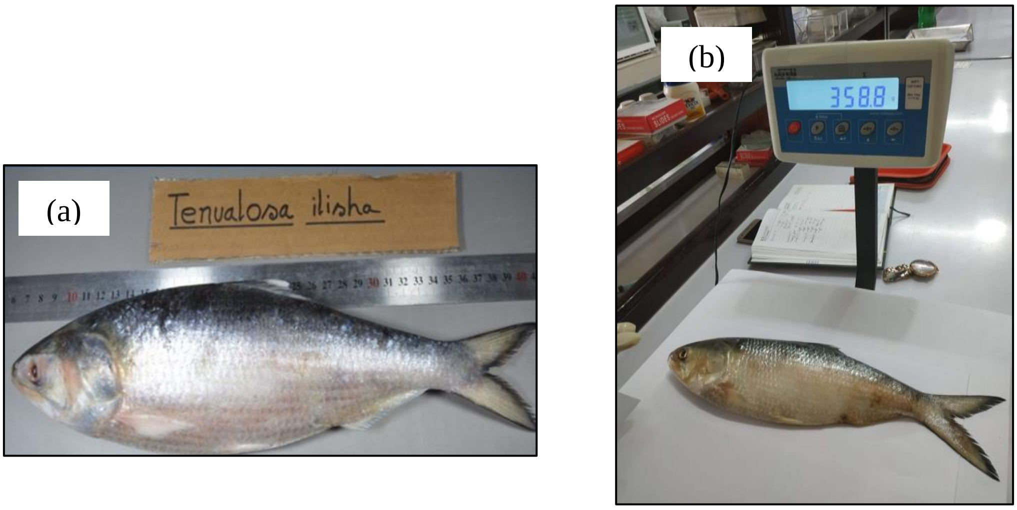


Fig. 06: a) Recording of length data and b) recording of weight data of *T. ilisha*

The length-weight relationship was calculated by the equation:

$$\mathbf{W = aL^b}$$

where W is the total body weight (g), L is the total length (cm), (a) is a coefficient related to the body form and (b) is exponent indicating isometric growth when equal to 3 (Wootton, 1990). a and b parameters were estimated by linear regression on the transformed equation:

$$\mathbf{Log10\ (W) = log10\ a + b * log10\ (L)}$$

3.5 Determination of condition factor

Condition factor (K) is considered as one of the most important factors influencing body composition. It is a measurement of the general health condition of fish as calculated by the ratio of body weight to body length. It is also often employed to measure the effects of environmental factors. Condition factor/Fulton's condition factor (Fulton, 1904) was estimated using following equation:

$$\mathbf{K = W*100/L^3}$$

Where,

W = Weight of fish in gram (g)

L = Length of fish in centimeter (cm)

3.6 Estimation of relative condition factor

The relative condition factor is the ratio of observed weight of a fish at a given length to the expected weight of a fish of the same length as calculated from the length-weight regression (Le Cren, 1951). Relative condition factor for each individual was calculated by using following equation:

$$\mathbf{K = W/a*L^b}$$

Where,

W = Weight of fish

L = Length of fish

a and b = Regression of co-effecient

3.7 Collection of gonad:

For characterizing the gonad development stages of hilsa gonads were collected from both sexes. After removing the unwanted dirt, digestive parts and intestine, the gonads were collected carefully by using forceps (Fig. 07).

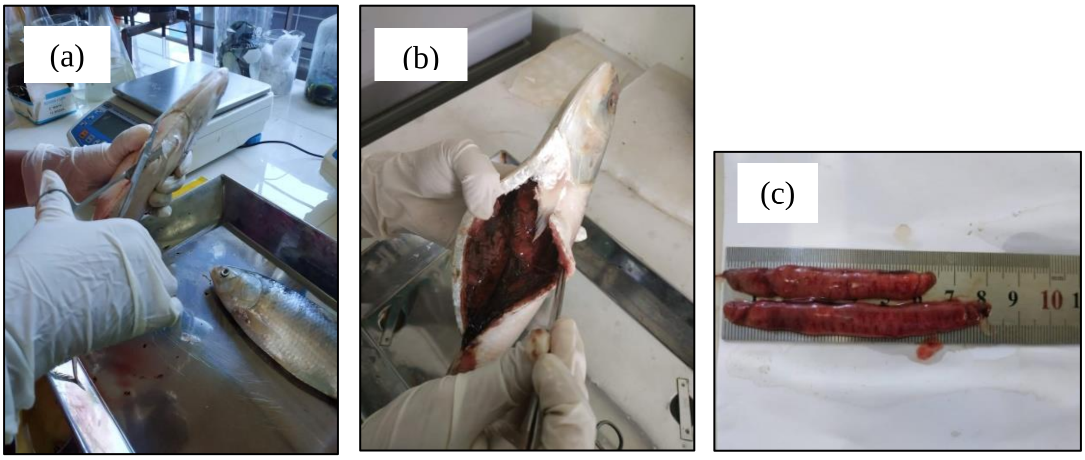


Fig. 07: a) Dissecting sample, b) Removing digestive part and c) Collected gonad

3.8 Estimation of fecundity

Gravimetric method was used to estimate the fecundity (Hunter *et al*., 1989). After the collection of gonad, excess water was removed with the blottiong paper. At last 3-4 subsamples from different part of the ovary was cut and then weighed the subsamples. After weighing, the numbers of eggs was counted (Fig. 08) for each sample and fecundity was determined by the following formula:

Fecundity = (Total ovary weight/Weight of sub-sample) × No. of eggs in sub-sample

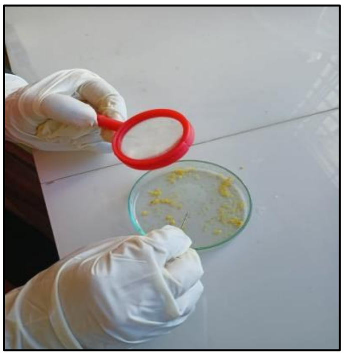


Fig. 08: Counting of eggs

3.9 Estimation of oocyte diameter

Diameter of the oocyte was measured in every month by ocular microscope in micrometer (Fig. 09).

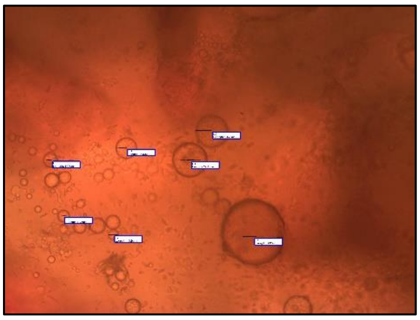


Fig. 09: Measurement of oocyte diameter (µm)

3.10 Gonadosomatic Index (GI):

To determine sexual maturity, gonads from fish sample were weighted and morphologically examined. In order to determine, Gonadosomatic Index (GSI) value for each month, were used according to the following formula:

$$\textbf{GSI (\%)} = \textbf{Gonad weight (g)} \times \textbf{100 / Body weight (g)}$$

The spawning seasons determined based on the monthly changes of GSI indices and proportions of each maturity stage (Zhang *et al.*, 2009).

3.11 Histological process:

Seasonal maturation of gonads was studied histologically to determine breeding season and maturation of fish. Techniques used for the preparation of histological permanent slide include processing of tissue, staining and mounting. Microscopic slides were prepared by following procedures:

3.11.1 Fixation:

A small part of the gonad from each dissected fish were sub-sampled by cutting a transverse section midway along the anteroposterior axis. Then gonads were placed in fixative for killing and hardening of tissue. In this study, Bouin's Solution was used as fixative and each gonad was kept in a vial filled up with fixative solution (Fig. 10).

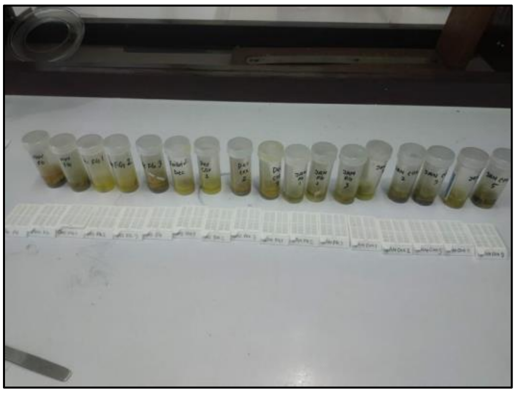


Fig. 10: Gonad samples in Bouin's fixative

3.11.2 Dehydration:

After washing, the cassettes with tissue blocks were passed through successive ascending concentration of alcohol for dehydration is described in table 01. Dehydration helps to provide ideal consistency of tissue for cutting in thin slice (Sectioning). As paraffin is not miscible with water, so water must be replaced by immersing in ascending concentration of alcohol (Fig. 11).

Table 01: Dehydration procedure for histological preparation of gonad samples

Sl No.	Treatment	Time
1	50% Alcohol	2 to 3 hours
2	70% Alcohol	2 to 3 hours
3	80% Alcohol	2 to 3 hours
4	90% Alcohol	2 to 3 hours
5	95% Alcohol	2 to 3 hours
6	100% Alcohol	2 to 3 hours
7	100% Alcohol	2 to 3 hours

Fig. 11: Dehydration of tissue blocks in alcohol

3.11.3 Cleaning:

After the alcohol treatment, blocks were passed through successive changes of xylene to replace the alcohol from tissue by xylene which is listed in table 02. To remove the opacity of dehydrated tissue and to make tissues clear, this step is essential (Fig.12).

Table 02: Cleaning procedure for histological preparation of gonad samples

Sl No.	Treatment	Time
1	50% Alcohol + 50% Xylene	2 hours
2	Xylene	2 hours
3	Xylene	2 hours

Fig. 12: Cleaning of tissue blocks

3.11.4 Infiltration:

After cleaning, tissue blocks were placed into melted paraffin in the oven at 60°c. This heat is required to evaporate xylene that helps in good infiltration of paraffin which is mentioned in table 03 with specific duration.

Table 03: Infiltration procedure for histological preparation of gonad samples

Steps	Treatment	Time
1	50% xylene + 50% Paraffin	2 hours or overnight
2	Paraffin	2 to 4 hours
3	Paraffin	2 to 4 hours
4	Paraffin	2 to 4 hours

3.11.5 Embedding:

The gonadal tissues were then embedded in paraffin blocks. After embedding the tissues, the paraffin blocks were trimmed to facilitate accurate sectioning (Fig. 13).

Fig. 13: Tissue embedding

3.11.6 Trimming:

Trimming is a process in which the undesirable wax layers of the embedded blocks are trimmed by knife to obtain suitable blocks. It helps for easy sectioning. After proper embedding, trimming was done to trim undesirable wax layers (Fig. 14).

Fig. 14: Trimming of unwanted paraffins

3.11.7 Sectioning:

Paraffin block with tissue was sectioned with hand rotatory microtome machine. Thickness of the tissue was about 5-6 µm. Sectioned tissue ribbon was placed in slide where 1-2 drops of alcohol was used on it and floated in water bath below melting temperature of paraffin (about 40^{o} c). When sectioned ribbon was well spread, they were placed into glass slide and tagged serially. Then those slides were dried with the help of slide warmer at 37°c for few hours (Fig. 15).

Fig. 15: Sectioning tissue in Microtome

3.11.8 Staining:

After drying, the slide with tissue section was stained by following the list which is mentioned in table 04.

Table 04: Hematoxylene and Eosin staining:

Sl No.	Solutions	Time	Process
1	Xylene	10 minutes	Clearing
2	Xylene	10 minutes	
3	Xylene	10 minutes	
4	100% alcohol	5 minutes	Rehydration
5	100% alcohol	5 minutes	
6	90% alcohol	3 minutes	
7	80% alcohol	3 minutes	
8	70% alcohol	3 minutes	
9	50% ethyl alcohol	2 minutes	Staining
10	Distilled water	15 dips	
11	Haematoxylene	3 minutes	
12	Wash in tap water	15 minutes	
13	50% ethyl alcohol	10-15 dips	
14	95% ethyl alcohol	30 seconds	
15	Eosin Y	1 minute	
16	95% ethyl alcohol	2 minutes	Dehydration
17	100% ethyl alcohol	1 minute	
18	100% ethyl alcohol	3 minutes	
19	100% ethyl alcohol	1 minute	
20	Xylene	20 minutes	Clearing
21	Xylene	20 minutes	
22	Drying at room temperature	Overnight	Drying

3.11.9 Mounting:

After completion of staining, mounting is essential to prepare permanent slide. One or two drops of DPX (mountant) were put on the dried slide which one was stained and dried. Then a cover slide was slowly lowered and the mountant flowed towards the descending glass without creating air bubbles. After mountant, Slides were kept overnight for dried and marked with marker pen (Fig. 16).

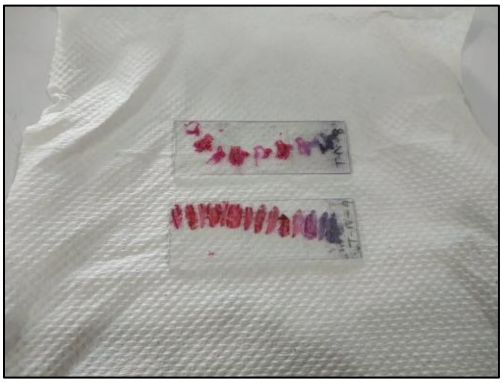


Fig. 16: Mounted slides

3.12 Microscopic observation:

The mounted slides were observed under a microscope, which was connected to computer with Digital camera. By the help of this mechanism several photographs were taken at different magnifications (Fig. 17).

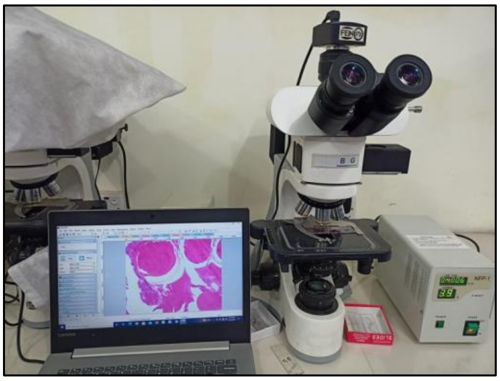


Fig. 17: Microscopic observation of sample slide

3.13 Data Analysis:

All statistical analysis and recording were performed using computer based software Microsoft Excel, 2013. Data were expressed as mean ± standard deviation (S.D.) of mean.

CHAPTER FOUR

RESULTS

The results section presents the finding of an investigation in a systematic, logical, concise and comprehensive manner. This section contains detailed information of the present research work about length-weight relationship, condition index, fecundity, oocyte diameter, GSI and histological changes in the gonads of *T. ilisha* throughout the year.

4.1 Length-weight relationship

Simple linear regression was used to identify the length-weight relationship (Fig. 18). Data value obtained for *T. ilisha* in this experiment to calculate the intercept (Log a) and slope (b) of the regression analysis were -1.3098 and 2.6351, respectively.

The length-weight relationship based on pooled data was W = 0.269874* TL $^{2.6351}$ (R^2 = 0.682), the logarithmic equation being log W = -1.3098 + 2.6351 logTL (Fig. 19). The 'b' value of the length-weight relationship indicates the negative allometric growth (b<3) of *T. ilisha*. The coefficient of determination (R^2 = 0.682) revealed that 68% of the variation in body weight due to variation in total length in the sample collected over the period.

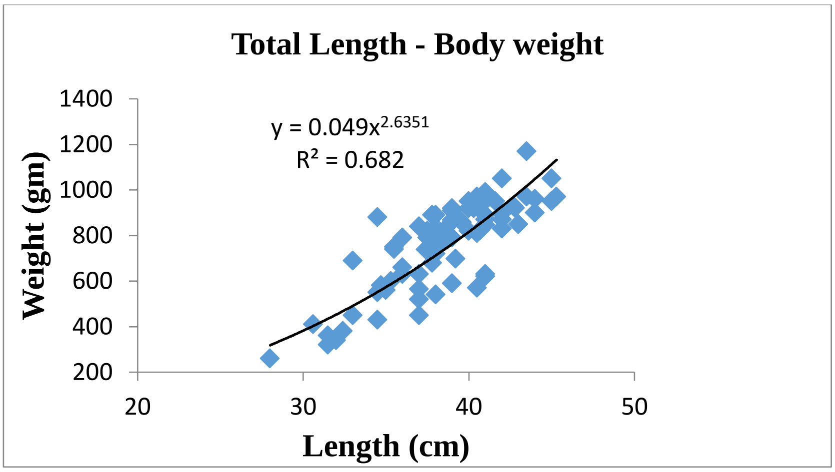


Fig. 18: Simple linear regression between length and weight dimensions of collected sample

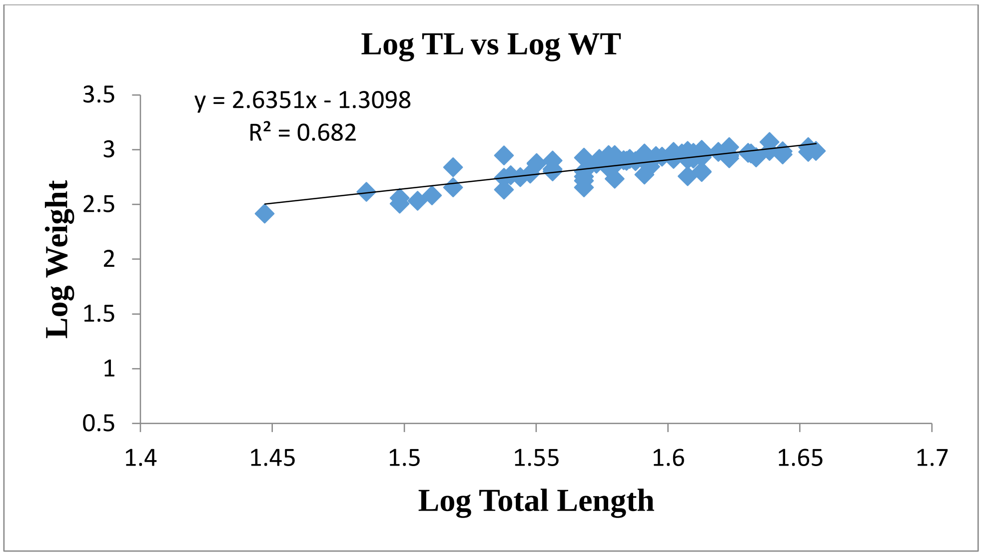


Fig. 19: Logarithmic relationship between the total length and weight based on pooled data of *T. ilisha*.

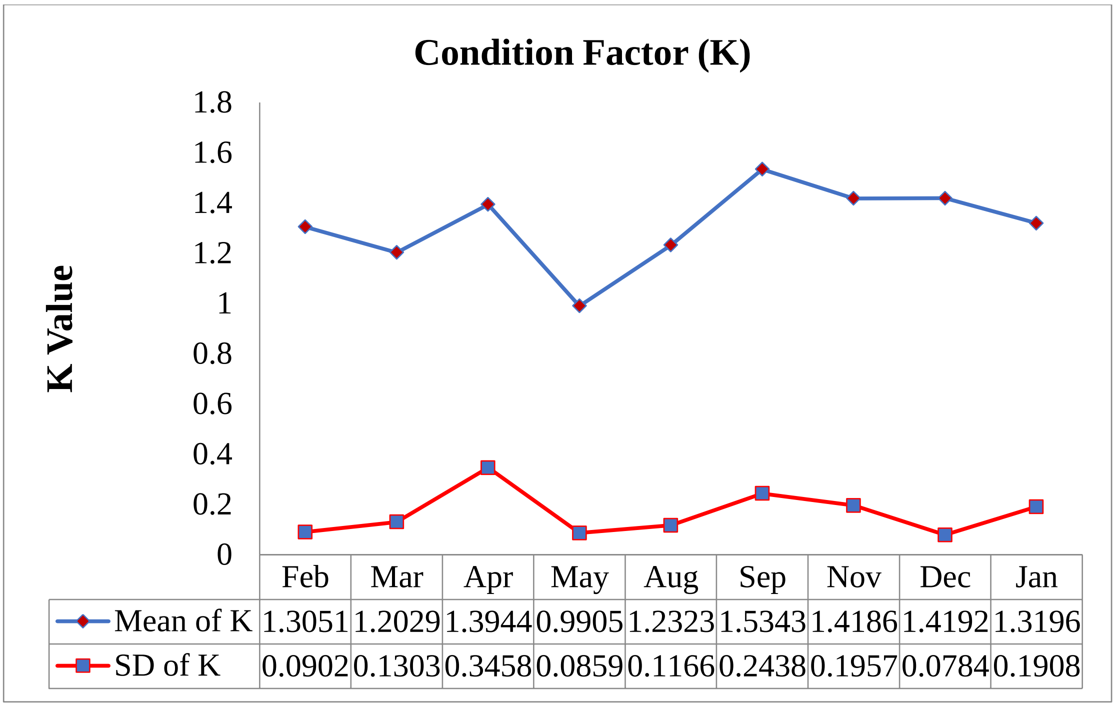
4.2 Condition factor and relative condition factor

Condition factor (K) and relative condition factor (K_n) were calculated in every month to observe the overall health condition of the fishes. Condition factor (K), measures the deviation of an individual from the average weight for length and relative condition factor (K_n), measures the deviation from the hypothetical ideal fish. The mean values of condition factor and relative condition factor, 'K' and 'K_n' for pooled data of T. ilisha were calculated for each months throughout the year is listed in table 05. The monthly mean K values obtained varied from 0.9905±0.0859 to 1.5343±0.2438 with an average value of 1.3129±0.1642 and the monthly mean K_n values obtained varied from 0.1404±0.0126 to 0.2177±0.0286 with an average value of 0.1842±0.0221. It was found that, the mean K was minimum (0.9905±0.0859) during May and maximum (1.5343±0.2438) during September (Fig. 20) and the mean K_n was minimum (0.1404±0.0126) during May and maximum (0.2177±0.0286) during September (Fig. 21).

Table 05: Condition factor and relative condition factor data of T. ilisha of 9 months. Data are presented as mean ± standard deviations for each month over the period from February, 2019 to January, 2020.

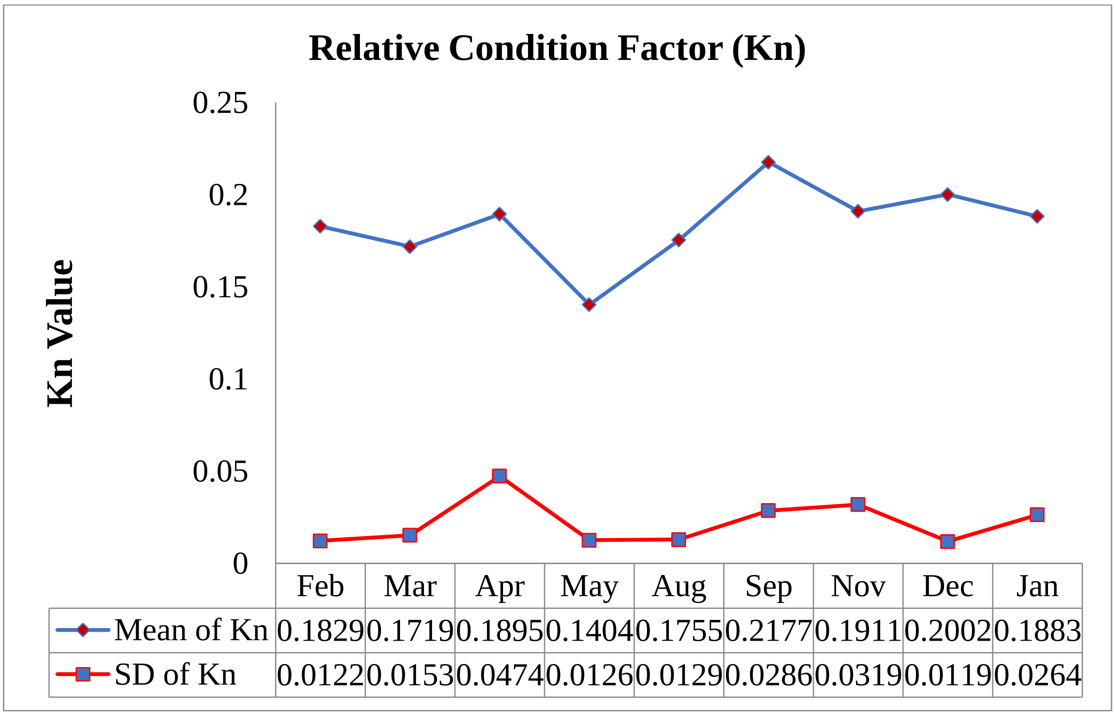
Month	Mean K ± S.D.	Mean K_n ± S.D.
February, 2019	1.3051±0.0902	0.1829±0.0122
March, 2019	1.2029±0.1303	0.1719±0.0153
April, 2019	1.3944±0.3458	0.1895±0.0474
May, 2019	0.9905±0.0859	0.1404±0.0126
August, 2019	1.2323±0.1166	0.1755±0.0129
September, 2019	1.5343±0.2438	0.2177±0.0286
November, 2019	1.4186±0.1957	0.1911±0.0319
December, 2019	1.4192±0.0784	0.2002±0.0119
January, 2020	1.3196±0.1908	0.1883±0.0264
Average	1.3129±0.1642	0.1842±0.0221

Month wise value of condition factor 'K' and relative condition factor 'K_n' were calculated and presented in the Figure-3 and Figure-4, respectively. This one year comparison showed that there was a similar trend between condition factor 'K' and relative condition factor 'K_n' throughout the year.



	Feb	Mar	Apr	May	Aug	Sep	Nov	Dec	Jan
Mean of K	1.3051	1.2029	1.3944	0.9905	1.2323	1.5343	1.4186	1.4192	1.3196
SD of K	0.0902	0.1303	0.3458	0.0859	0.1166	0.2438	0.1957	0.0784	0.1908

Fig. 20: Condition factor based on pooled data of *T. ilisha* of 9 months. Data are presented as mean ± standard deviations for each month over the period.



	Feb	Mar	Apr	May	Aug	Sep	Nov	Dec	Jan
Mean of Kn	0.1829	0.1719	0.1895	0.1404	0.1755	0.2177	0.1911	0.2002	0.1883
SD of Kn	0.0122	0.0153	0.0474	0.0126	0.0129	0.0286	0.0319	0.0119	0.0264

Fig. 21: Relative condition factor based on pooled data of *T. ilisha* of 9 months. Data are presented as mean ± standard deviations for each month over the period

4.3 Gonado Somatic Index (GSI)

In this study all samples were taken into consideration for calculation of GSI throughout the year. The Gonado Somatic Index for each fish was calculated and averaged for each month.

Highest GSI value (13.57±1.618) for female was found in January whereas lowest value of GSI (1.698±0.4968) was observed in March. Another high value of GSI was also observed in September (10.11±0.835) which indicates the second phase of ovarian maturation in female. Mean GSI value of female *T. ilisha* increased significantly from the month of March to May. After that June and July was the ban period for fishing. Then GSI value increased again in September and October is the ban period for hilsa fishing. In the month of January GSI value reached at the peak (Fig. 22).

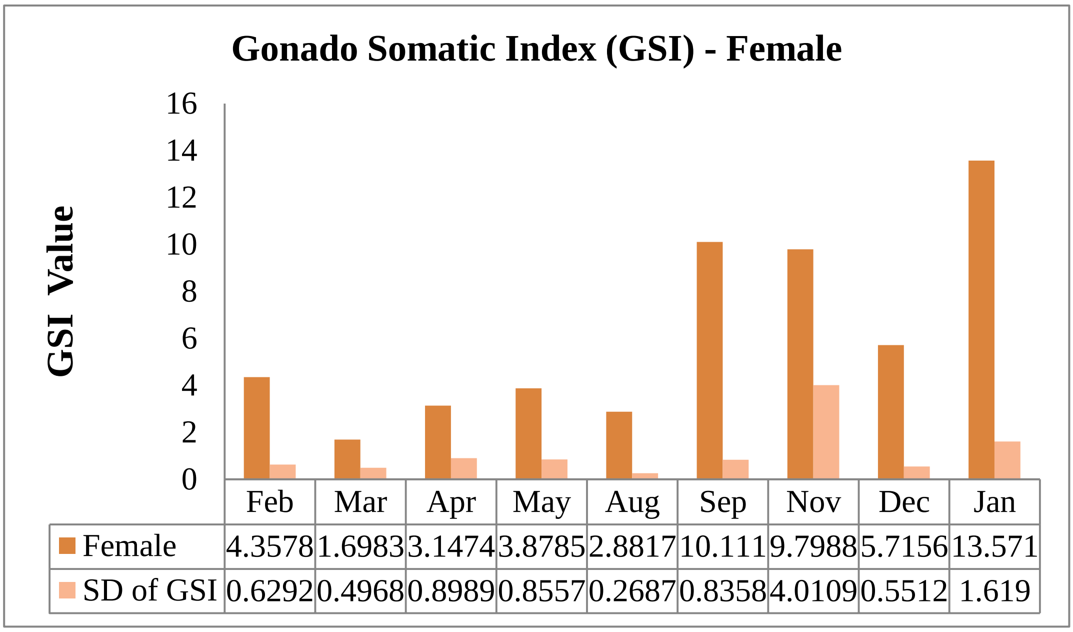
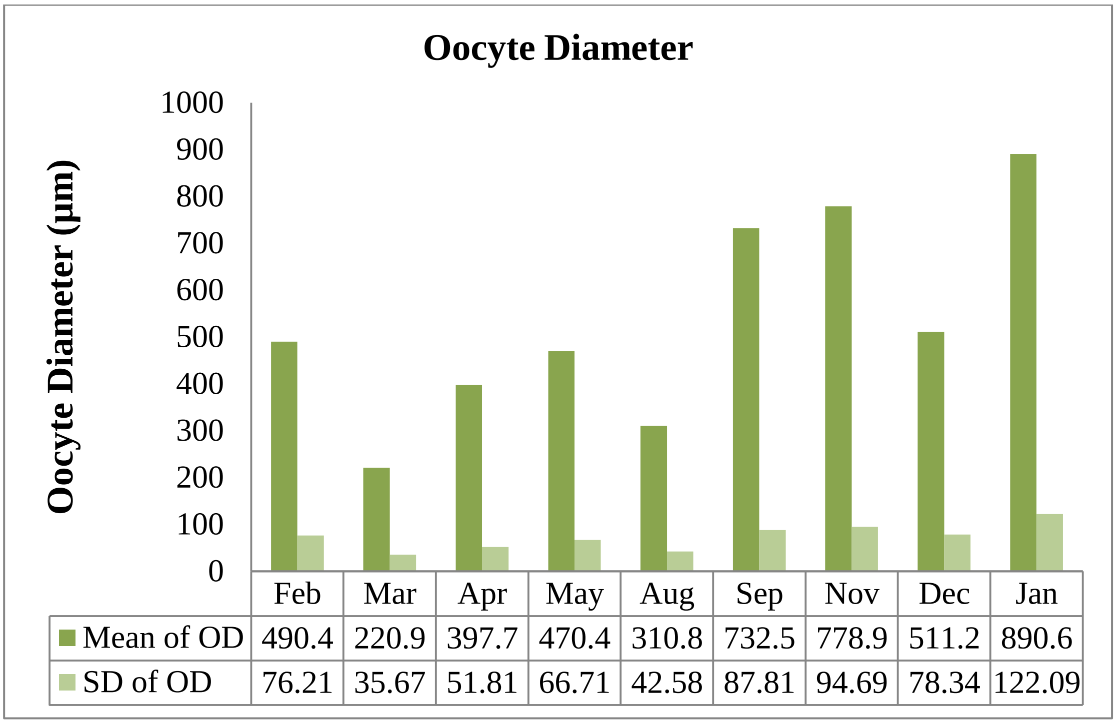


Fig. 22: Monthly variation of gonado somatic index (GSI) in female *T. ilisha*. Data are presented as mean ± standard deviations for each month over the period

4.4 Oocyte Diameter

During investigation annual increase in the oocyte diameter of *T. ilisha* was observed every month throughout the year and compared with the GSI of females. Data of oocyte diameter showed significant correlation with the GSI changes, which suggest a synchronize development of gonads with the increasing size of eggs. Maximum oocyte diameter was found in January (890.6±122.09 µm) whereas minimum oocyte diameter was found in March (220.9±35.67 µm) (Fig. 23).



	Feb	Mar	Apr	May	Aug	Sep	Nov	Dec	Jan
Mean of OD	490.4	220.9	397.7	470.4	310.8	732.5	778.9	511.2	890.6
SD of OD	76.21	35.67	51.81	66.71	42.58	87.81	94.69	78.34	122.09

Fig. 23: Oocyte diameter of *T. ilisha* collected from Chattogram coast. Data are presented as mean ± SD

4.5 Fecundity

Fecundity is one of the most essential features of fish reproductive biology since it tells us how many mature eggs a female may lay in a single spawning season. The fecundity was determined from randomly collected gravid female ranging from 34.5 cm (430gm) to 45.3 cm (970gm) in total length has ovaries weighing from 15.35 to 19.5g. Fecundity was counted by gravimetric methods and was found to vary from 96718 to 117435 with a mean value of 109605±1635 eggs.

4.6 Gonadal maturation stages in female

Throughout the year the gross ovarian stages of *T. ilisha* and monthly ovarian development were investigated. Each of the histological ovarian section was studied thoroughly and described the different stages of ovarian maturation.

The following stages of ovarian development were identified in *T. ilisha*:

4.6.1 Primary vitellogenic stage:

This stage is often called the early maturing stage of the oocyte. Ovary increases in size, pale-yellow to dark pink in color and occupies about half of the peritoneal cavity. In this stage ovary filled with early perinucleolar oocyte (Epo) and late perinucleolar stage oocytes (Lpo). Blood capillaries in this stage were still not clear. This stage was found in August (Fig. 24).

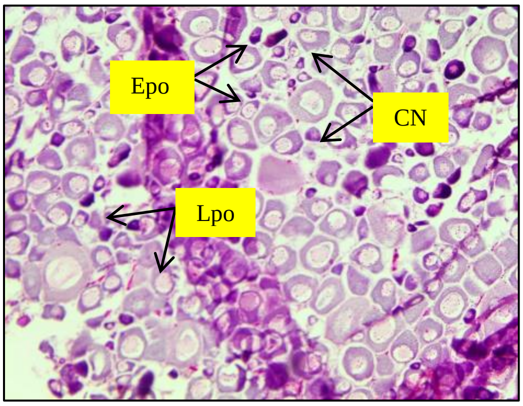


Fig. 24: Primary vitellogenic stage of ovarian development of *T. ilisha*. Here, CN- Chromatin nucleolar, Epo- Early perinucleolar oocyte, Lpo- Late perinucleolar oocyte

4.6.2 Secondary vitellogenic stage:

The secondary vitellogenic stage is accompanied with the accumulation of eosiniphilic yolk globule in the inner cortex. The cytoplasm was mostly covered with yolk globules sometimes with large oil droplets. The nucleus contains some peripheral nucleoli. Thick zona radiate was observed. Blood vessel was appeared in the surface of the ovary. The number of ova can be counted by naked eye. The stage was found during the month of September and November (Fig. 25).

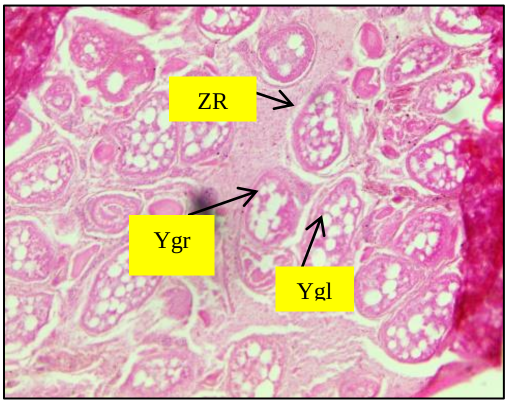


Fig. 25: Secondary vitellogenic stage of *T. ilisha*. Here, GV- Germinal vesicle, ZR- Zona radiate, Ygr- Yolk granule, Ygl- Yolk globule

4.6.3 Maturing stage:

In this stage perinuclear oocyte and yolk vesicle was visibled clearly. The stage was found in April and May (Fig. 26).

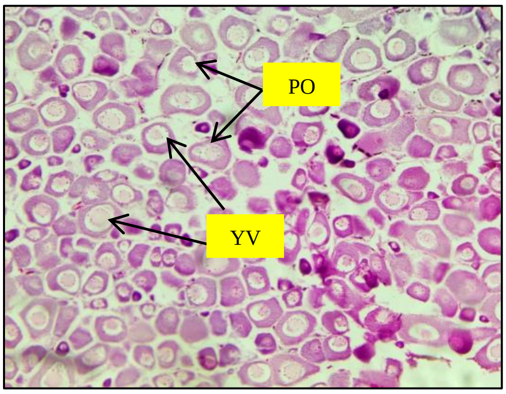


Fig. 26: Maturing stage of *T. ilisha*. Here PO- Perinuclear oocyte and YV- Yolk vesicle

4.6.4 Ripe stage:

Ovary was swollen, maximally distended and yellowish, occupying majority of the body cavity. Eggs were clearly visible throughout the thin ovary wall. A network of blood vessels surrounds the organ. Post-ovulatory follicle often co-existed with the yolk granules in this stage. Ovary was fully large in this stage, yellowish and orange in color and occupied the entire body cavity. This stage was found in January (Fig. 27).

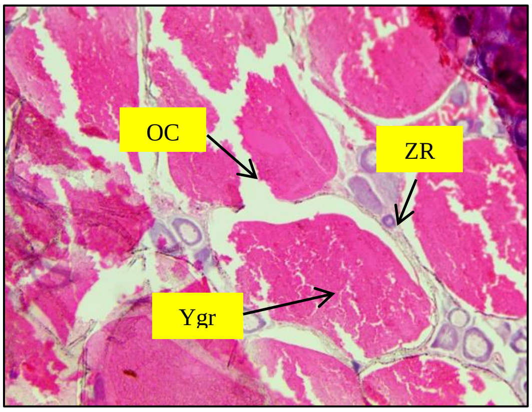


Fig. 27: Ripe stage of *T. ilisha*. Here, ZR- Zona radiata, Ygr- Yolk granule and OC- Oocyte cavity

4.6.5 Spent:

The weight of the ovary drastically became low due to release of eggs. Ovary was reddish and flaccid, shrunken, hollow, sac like structure, small number of immature eggs were present sometimes with denatured ova. This stage has found in February (Fig. 28).

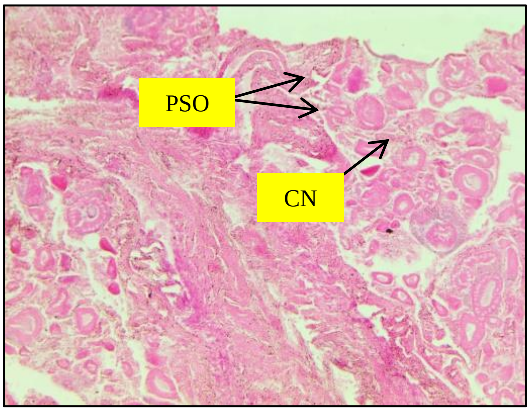


Fig. 28: Spent stage of *T. ilisha*. Here, CN- Chromatin nuclear and Pso- Partially spent ovary

4.6.6 Regressing stage:

Ovary of totally spent females contain numerous post-ovulatory follicles at different stage of degeneration, atretic oocytes and a reserve stock of oogonia and perinucleolar stage oocytes. This stage has found in March (Fig. 29).

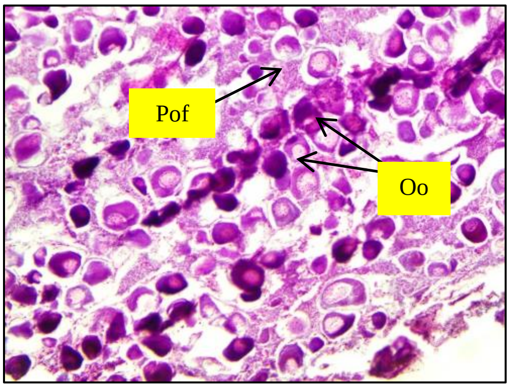


Fig. 29: Regressing stage of *T. ilisha*. Here, Pof- Post ovulatory follicle and Oo- Oogonia

CHAPTER FIVE

DISCUSSION

This research work was designed to describe breeding biology of *T. ilisha*. This chapter described the overall results comparison, achievement of the study and problems associated to the research workon the reproductive aspects of *T. ilisha.*

5.1 Length-weight relationship

In this study simple linear regression was used to identify the length-weight relationship. Data value obtained for *T. ilisha* in this experiment to calculate the intercept (Log a) and slope (b) of the regression analysis were -1.3098 and 2.6351, respectively. The length-weight relationship based on pooled data was W = 0.269974* TL $^{2.6351}$ (R^2 = 0.682), the logarithmic equation being log W = -1.3088 + 2.6361 logTL. In the study the slope 'b' value was less than 3 which indicates that *T. ilisha* become more slender as they increase in length and the growth pattern was allometric, not isometric.

Halim *et al.* (2020) has also recorded the allometric growth of *T. ilisha* in the mouth of Perak waters, Malaysia. He recorded the exponent b value 2.8067 for female *T. ilisha*, which is less than 3 and indicating an allometric relationship. However, Flura *et al*. (2015) reported isometric growth in Meghna river, Bangladesh with the b value 3.079.

5.2 Condition factor and relative condition factor

In this study the mean values of condition factor and relative condition factor, 'K' and 'K_n' for pooled data of *T. ilisha* were calculated for each months throughout the year. The monthly mean K values obtained varied from 0.9905±0.0859 to 1.5343±0.2438 with an average value of 1.3139±0.1643 and the monthly mean K_n values obtained varied from 0.1404±0.0126 to 0.2167±0.0287 with an average value of 0.1843±0.0222. It was found that, the mean K was minimum (0.9905±0.0859) during May and maximum (1.5344±0.2439) during September and the mean K_n was minimum (0.1405±0.0127) during May and maximum (0.2177±0.0286) during September. This one year comparison showed that there was a similar trend between condition factor 'K' and relative condition factor 'K_n' throughout the year.

Hunter *et al.* (1989) estimated the Fulton's condition factor and relative condition factor , showed that there was no apparent correlation between GSI and condition factors.

Kumar and Benerjee (2012) observed the relative condition factor ranged from 0.98±0.14 to 1.03±0.17 for *T. ilisha* in Hoogly estuarine.

5.3 Gonado-somatic index (GSI)

In the study highest GSI value (13.57±1.618) for female was found in January whereas lowest value of GSI (1.699±0.4968) was observed in March. Another high value of GSI was also observed in September (10.12±0.836) which indicates the second phase of ovarian maturation in female. Mean GSI value of female *T. ilisha* increased significantly from the month of March to May. After that June and July was the ban period for fishing. Then in the month of September GSI value increased again and October is the ban period for Hilsa fishing. In the month of January GSI value reached at the peak and then drastically decreased.

Hunter *et al.* (1989) reported that the spawning in females started in April and gradually increased till October. They were observed peak in October, whereas in April the lowest GSI value was recorded. Again Halim *et al.* (2020) revealed that spawning season for female *T. ilisha* is from April to May.

5.4 Oocyte diameter

In this study, annual increase in the oocyte diameter of *T. ilisha* was observed every month throughout the year and compared with the GSI of females. Data of oocyte diameter shows significant correlation with the GSI changes, which suggest a synchronize development of gonads with the increasing size of eggs. Maximum oocyte diameter was found in January (890.7±122.08 μm) and minimum oocyte diameter was found in March (220.8±35.68 μm).

Almukhter *et al.* (2016) mentioned that the strong relationship between egg size and GSI was expected because GSI increases with egg maturity.

5.5 Fecundity

The fecundity was determined from randomly collected gravid female ranging from 34.5 cm (430g weight) to 45.3 cm (970g weight) in total length has ovaries weighing from 15.34 to 19.6g respectively. Fecundity was counted by gravimetric methods and was found to vary from 96718 to 117535 with a mean value of 109605±1635 eggs. According to Roomiani *et al*. (2014), the average facundity of *T. ilisha* is 113,483, 382,106, and 572,708 eggs per size class of 200-250 mm, 250-300 mm, and 301-350 mm, respectively. The facundity of *T. ilisha* was found to be between 223,750 to 1,477,790 oocytes in a research conducted in the Southern Al Hammar Marsh in Basra, Iraq by Almukhtar *et al*. (2016). When comparing the fecundity values recorded in this study to those published in earlier studies, the size of the fish had a significant impact on fecundity.

5.6 Histological observation of gonad

Six development stages were found in the collected female samples from the Chattogram coast. Early vitellogenic stage was found in August when ovary increases in size, pale-yellow to dark pink in color and occupies about half of the peritoneal cavity. The secondary vitellogenic stage is accompanied with the accumulation of eosiniphilic yolk globule in the inner cortex which was observed in September and November. In the month of April and May maturing stage was observed. In December and January ripe stage was found when eggs were clearly visible throughout the thin ovary wall and ovary was swollen, maximally distended and yellowish. Finally, the stage of spent and regressing were found in February and March, respectively. Almukhtar *et al*. (2016) reported that *T. ilisha* has an extended spawning periodicity in the mouth of Perak waters, Malaysia, beginning in February and ending in September. Roomiani *et al.* (2014) informed that *T. ilisha* in coastal waters of the Northwest of Persian Gulf has a spawning period from March to June.

CHAPTER SIX

CONCLUSION

The present study has been carried out with an aim to describe the spawning season and life history characteristics of *T. ilisha* along the Chattogram coast, Bangladesh. Utilizing the data gathered, several analyses were carried out in order to determine the end outcome of the research. For analyzing the reproductive pattern and histological profile of *T. ilisha* in this study length-weight relationship, condition factor, relative condition factor, oocyte diameter, fecundity and histological stages were investigated. Six gonadal development stages have found during the study. This study was done during the hydrological year of February, 2019 to January, 2020 but during this period June, July and October was the ban period. All the sections are described with logical data which is helpful for understanding the findings of the research. This research indicates that the GSI value is highest in January (13.57) and lowest in March (1.698). According to the GSI values, *T. ilisha* reaches its ripe stage in January and enters its regressing stage in March. It also goes through other phases including primary and secondary vitellogenic, maturing, and spent. Due to the spawning season, oocyte diameter is also reported to be at its maximum (890.6) and lowest (220.9) values in January and March, respectively. For the different sample sizes, fecundity was also evaluated with a mean value of 109605±1635 eggs, ranging from 96718 to 117435.

CHAPTER SEVEN

RECOMMENDATION AND FUTURE PERSPECTIVES

The Hilsa fishery is directly or indirectly responsible for the livelihood of a huge number of people in our country. The ultimate goal of this study is to ascertain the reproductive biology of *Tenualosa ilisha* from the Chattogram coast. In this study histological profile is also identified. This study provides the baseline information about the spawning season of *T. ilisha*. The study includes several other drawbacks as well. We are all aware of how expensive Hilsa is and because of its exorbitant cost a large sample size for my study is not feasible. The outcome may be more accurate if the sample size was large. Despite these limitations the result of the research will assist people to avoid catching Hilsa throughout the spawning season. The gonadal stages of the Hilsa may be easily spotted by distinguishing the histological profile, which will help to clarify which month is acceptable for catching and which is not. People may be informed about this issue as well as the government can be strict for implementing the laws. Thus we can protect this wealth of our country and livelihood can be maintained. As Hilsa is also exported for earning foreign currency this study will help to conserve the female Hilsa at breeding period and finally increase the total production.

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Appendix A

Average and SD value of GIS according to the month:

Month	Average GIS value	S.D. of GIS
February	4.357806606	0.62919
March	1.698257792	0.4968
April	3.147440319	0.898945
May	3.8785057	0.855719
August	2.881681825	0.268714
September	10.11097175	0.835754
November	9.798838384	4.010854
December	5.715627927	0.551175
January	13.57108194	1.618965

Appendix B

Mean and SD of the Condition Factor:

Month	Mean of CF	S.D. of CF
February	1.3050722	0.0902522
March	1.2029981	0.1302528
April	1.3944296	0.3458446
May	0.9904805	0.0859085
August	1.2322903	0.1166362
September	1.5342512	0.2437936
November	1.4185649	0.1956825
December	1.4191906	0.0783828
January	1.3196414	0.1908054

Appendix C

Mean and SD of the Relative Condition Factor:

Month	Mean of RCF	S.D. of RCF
February	0.182990107	0.01222
March	0.171973573	0.015301
April	0.189486113	0.04744
May	0.140351378	0.012562
August	0.175530136	0.0129
September	0.217667853	0.028551
November	0.191065439	0.031876
December	0.200168555	0.01199
January	0.188264833	0.026374

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